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Glenda Carrasquillo

Glenda Carrasquillo

Docket No.: 515-4218

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

PATENT OPERATIONS

In re Application of:)
)
Achille Arini et al.) Group Art Unit: 1652
)
) Examiner: Steadman, David J.
Serial No.: 09/815,533)
)
Filed: March 16, 2001)
)

For: **METHOD FOR THE PRODUCTION OF PHARMACEUTICALLY ACTIVE RECOMBINANT PROTEINS**

DRAFT CLAIMS

93. (new) A process for the production of recombinant two chain urokinase (tc-uPA) into the culture medium of an eukaryotic cell line wherein at least 95% of the total urokinase is catalitically active two chain urokinase (tc-uPA), said process comprising the following steps:
a) culturing a mammalian cell line which has been genetically manipulated with a cDNA sequence encoding for a urokinase precursor in a culture media comprising an alkanoic acid selected from the group consisting of: butyric acid, sodium butyrate sodium propionate, magnesium butyrate, tributyrin and phenyl-butyrate, their derivatives or salts thereof,
b) continuing said culture for a time of at least 120 hours.

94. A process according to claim 93 further comprising the recovery of the cell culture supernatant for the isolation of recombinant human tc-uPA.

95. A process according to claim 93 wherein said eukaryotic cell line is selected from CHO and CHO-Messi.

96. A process according to claim 93 wherein said cell culture medium is serum-free.

97. A process according to claim 93 wherein the concentration of said alkanoic acids is from 0.1 to 20 mM.

98. A process according to claim 93 wherein after said alkanoic acids are added, the cell culture is continued at a temperature from 30°C to 37°C.

99. A process according to claim 98 wherein said temperature is from 33°C to 35°C.

100. A process according to claim 94 wherein the tc-uPA in said cell culture medium is at least 4000iU/ml.

101. A process according to claim 94 wherein, the recovered culture medium is acidified with a weak acid to pH form 5.0 to 5.8, optionally a non-ionic detergent is added adn the culture medium is trhen filtered.